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DEVELOPMENT OF EGGS AND YOLK-SAC LARVAE OF YELLOWFIN MENHADEN

By JOHN W. REINTJES

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ABSTRACT

Fertilized eggs were obtained by manually mixing ova and sperm of yellowfin menhaden (*Brevoortia smithi*). Rearing was done in February 1960 at Indian River, Florida. Descriptions and illustrations are given for the developmental stages of the embryo and larva, through absorption of the yolk. Temperature and salinity observations are included.

DEVELOPMENT OF EGGS AND YOLK-SAC LARVAE OF YELLOWFIN MENHADEN

By JOHN W. REINTJES, *Fishery Research Biologist*
BUREAU OF COMMERCIAL FISHERIES

ıe menhadens, genus *Brevoortia*, inhabit the ıal waters of the western Atlantic Ocean from ı Scotia to central Argentina and support the st commercial fishery in the United States, their early developmental stages are little vn. Kuntz and Radcliffe (1917) described de- ɔing eggs, yolk-sac larvae, and older larvae ɔified as Atlantic menhaden (*B. tyrannus*). d on their descriptions, Atlantic menhaden and larvae have been reported from Chesa- e Bay (Pearson, 1941), Long Island Sound mutter, 1939; Wheatland, 1956; Richards,), and Narragansett Bay (Herman, 1959). , tentatively identified as Atlantic menhaden, obtained off the North Carolina coast in No- ɔer and December, 1956 and 1957 (Reintjes).[1] 957 eggs were hatched in the laboratory, but arvae died after the yolk sac was absorbed.

enhaden eggs and larvae were reported from ɪton collections made off the south Atlantic of the United States during three cruises of notorship *Theodore N. Gill* (Reintjes, 1961), no identification to species was made. Al- gh the foregoing observations provided a de- tion of eggs and larvae and information on distribution, some question remained as to her these actually were menhaden.

ıe absence of spawning, or running-ripe, fish e landings has precluded mechanical fertiliza- and rearing of the eggs and yolk-sac larvae he identification of Atlantic menhaden (*B. nus*) and Gulf menhaden (*B. patronus*), the species of principal commercial importance. occurrence of spawning yellowfin menhaden *mithi*) in the landings of a gill-net fishery at stian, Fla., made possible the distinction of and yolk-sac larvae of this species from those of other clupeoid fishes. Development of embryos and larvae was followed and described from the time of fertilization until absorption of the yolk.

The procedures of the work were: (1) obtain ripe ova and sperm from freshly caught yellowfin menhaden, (2) effect fertilization by mixing the sex products, (3) hold fertilized eggs in a suitable environment at known temperature during development, (4) remove and preserve examples of developing eggs and larvae, (5) observe the properties of eggs and the behavior of early larvae, and (6) collect planktonic eggs and larvae concurrently for comparative material.

MATERIALS AND METHODS

Beginning in November 1959 weekly samples of adult yellowfin menhaden were obtained from gill-net landings at Sebastian, Fla., to follow maturation of ovaries and testes. Each sample consisted of about 100 fish taken at random from the catch. Free-flowing milt was observed from cut testes in mid-December, and on January 11, 1960, several females in the sample extruded ova when pressed firmly. Each week thereafter, the number of fish apparently ready to spawn increased. On February 8, approximately one-fourth of the females and all of the males appeared ready to spawn.

On February 12, a temporary field laboratory was set up in a small dockside building at Sebastian, Fla. Equipment included compound and dissecting microscopes, thermometers, salinometers, small dip nets, one-half-meter plankton nets, an assortment of glass preparation bowls and polyethylene containers, and pens with nylon-net compartments. Other than the pens, no other equipment of special construction was used.

For rearing purposes, two pens, or enclosures, were constructed, following the design of the blue crab shedding floats, or live-cars, used throughout the Chesapeake Bay and middle Atlantic region

s and yolk-sac larvae of Atlantic menhaden. Unpublished manu- U.S. Bureau of Commercial Fisheries Biological Laboratory, Beau- C.

E.—Approved for publication June 28, 1961. Fishery Bulletin 202.

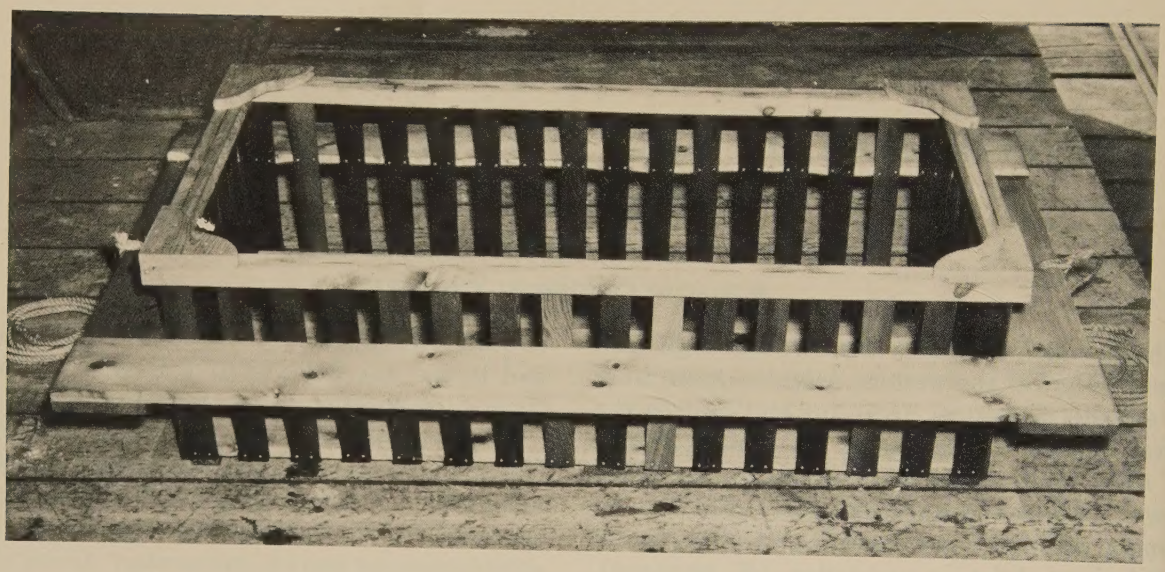


FIGURE 1.—Pen used to confine yellowfin menhaden eggs during development.

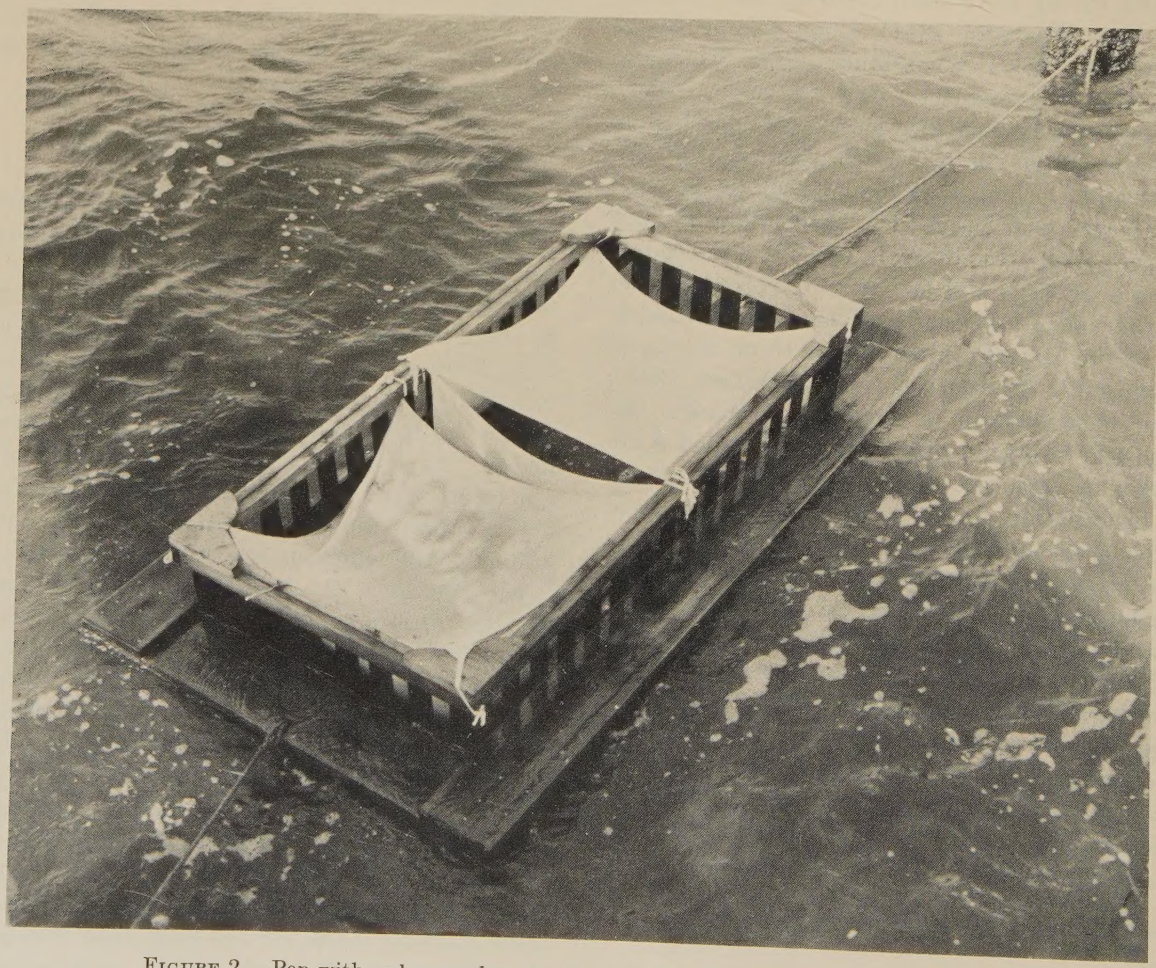


FIGURE 2.—Pen with nylon-mesh compartments floating in Indian River, Fla.

arton, 1954). The pens were made of juniper
ds and cedar slats (fig. 1). The dimensions
as follows: 18 inches high, 62 inches long,
32 inches wide, with a 5-inch flange on all
sides. The flange regulated the submergence
n of the pen and provided stability.
mpartments, made of woven-mesh nylon
ng, were used to confine the fertilized eggs
larvae within the pens. Two compartments
made of mesh with an average opening of
nm., and two compartments were made of
with an average opening of 1.0 mm.[2] All
s reinforced with nylon binding tape. The
partments were enclosed, except for access
one side (fig. 2). Because the access slit
d during rough weather and allowed debris to
and eggs to escape, a plastic slide fastener
was added. Each pen held two compart-
s that were kept in place by a tie at each
er.

llowfin menhaden used in the fertilization
were obtained from gill-net catches made
n sight of the temporary field laboratory.
lly, the nets were set at dusk and picked up
t an hour later. Ripe females with extruded
or greatly distended abdomens, were removed
set aside while the net was being recovered.
s producing milt with viable sperm commonly
red in the catch. Ripe females, on the
hand, were rare, for only 25 gravid females
found among approximately 4,000 fish ex-
ed. For all attempted fertilizations, fish
dead less than two hours.
rtilization was accomplished by mixing ova
sperm obtained by pressing the sides of the
or by cutting open the ovary or testes to
he mature sex products. The ova and sperm
mixed "dry," i.e., without sea water. Dock-
water was filtered through cotton to remove
isms including fish eggs and then added.
criterion of fertilization was the formation
vide perivitelline space.
rtilized eggs were placed in the mesh com-
nents of the floating pens and in glass or
thylene containers in the laboratory. Sam-
of the developing embryos were removed
dically from the floating pens and placed in
laboratory containers. Development was

[2] hundred percent Dupont nylon pattern No. 109 (0.5 mm.) and pat-
1400 (1.0 mm.).

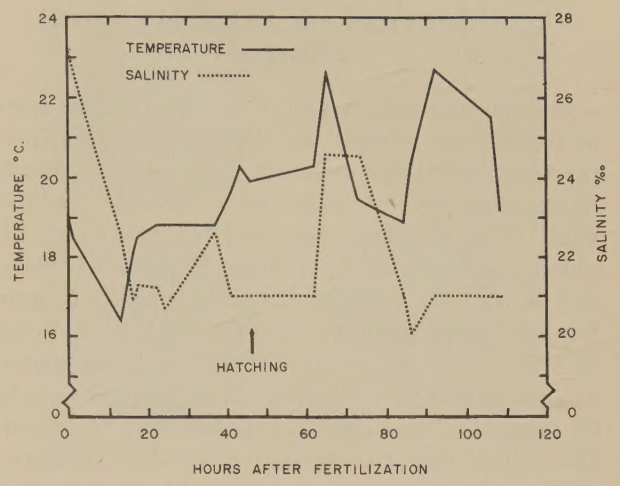


FIGURE 3.—Fluctuations of temperature and salinity during the development of eggs and yolk-sac larvae of yellowfin menhaden.

observed with a microscope, and samples were removed and preserved in 5 percent formalin.

The time required for development of the embryo was recorded as age-in-hours from manual fertilization and for the yolk-sac larva, from the time of hatching. The water temperature of Indian River, immediately adjacent to the dock and rearing floats, and of the culture bowls was recorded at infrequent intervals during development (fig. 3). The observed temperature ranged from 16.4° C. to 22.7° C., with a mean of 19.6° C.

Salinity was determined at each temperature observation. The observed salinity ranged from 20.1°/oo to 27.2°/oo, with a mean of 22.1°/oo.

Plankton collections of yellowfin menhaden eggs and larvae developing under natural conditions were obtained in the Indian River. Ten-minute tows were made with a half-meter net in the vicinity of the gill-net fishing grounds near Sebastian Inlet, and at one mile intervals for a distance of 6 miles north and 12 miles south of the inlet. Developing eggs from the plankton collections were used for the photographs of several stages not obtained during the development of artificially fertilized eggs. Although the size and appearance were similar to eggs of known origin, there were slight differences that are without adequate explanation. However, the identity of the planktonic eggs was assumed because of structural similarities and the concurrence of spawning yellowfin menhaden in the immediate vicinity.

DESCRIPTION OF FERTILIZATION TRIALS

Sixteen manual, or artificial, fertilizations were attempted to obtain developing embryos from positively identified yellowfin menhaden. A single female and several males were used in the first trial. The ova were removed by dissection, divided into three lots, and those in each lot mixed dry with milt from a separate male. Fifteen minutes later, filtered dockside water (salinity 20.5°/oo, and temperature 20.1° C.) was added to each container. An hour later, approximately 90 percent of the eggs in one lot were fertilized. The other two lots contained so few fertilized eggs, perhaps because of less viable sperm, that they were discarded. Development was arrested after several hours during early cleavage. Whether the failure to develop was due to decomposition, stagnation, or immaturity of ova or sperm could not be determined.

Four females were used in the second trial. Ova were removed by dissection, mixed "dry" with milt, and 15 minutes later, dockside water was added (27.2°/oo, 19.0° C.). The apparent success of fertilization varied from 40 to less than 10 percent. Two lots of eggs were placed in the floating pen anchored off the end of the dock where salinity was 26.7°/oo and temperature 18.5° C. The remaining two lots were placed in containers in the laboratory. Twelve hours later, eggs in the laboratory containers had failed to develop beyond early cleavage and showed signs of decomposition. Samples of eggs from the pens appeared normal, although in one compartment, few ova were fertilized. This trial furnished most of the developing embryos and yolk-sac larvae used for the descriptions.

DESCRIPTION OF EGG

Living eggs showed an iridescent, glasslike transparency, with little or no color in the yolk. Iridescence disappeared when the material was placed in formalin, but the chromatophores were retained and accentuated as the developing embryo and yolk became clouded. The following description is based on preserved material.

The egg is spherical and has a resilient, transparent membrane. Under magnification of 100 diameters or more, the membrane surface is marked with fine, short lines that form no discernible pattern. The yolk is segmented, contains a single oil globule, and is pale yellow. The oil globule is near the vegetative pole and floats uppermost throughout development. Coarse granulation of the yolk appeared to be characteristic of eggs not fully matured.

Comparative measurements showed the planktonic eggs to be slightly larger than those obtained artificially (table 1). Fertilized eggs in the plankton, similar in appearance and structure to those artificially fertilized, were assumed to be from yellowfin menhaden. Whether the artificially fertilized eggs had not reached maximum size because of immaturity, or whether naturally spawned eggs swell to a greater size could not be determined. Eggs, ranging from approximately 1.0 to 1.1 mm. in diameter, developed a fertilization membrane and perivitelline space; however, the very low fertility and the failure of most eggs to develop beyond the earliest stages of cleavage indicated that these ova had not reached maturity.

TABLE 1.—*Measurements of yellowfin menhaden eggs, in millimeters*

Item	Planktonic eggs (N=200)		Artificially fertilized eggs (N=50)	
	Range	Mean	Range	Mean
Fertilized egg	1.21–1.48	1.34	1.15–1.30	1.22
Perivitelline space	.33– .50	.42	.34– .46	.39
Yolk	.77–1.04	.90	.77– .95	.86
Oil globule	.05– .18	.13	.07– .16	.13

Developing yellowfin menhaden eggs from the plankton were buoyant, floating just beneath the surface film. Unfertilized eggs rested on the bottom in still water. Artificially fertilized eggs formed a layer above the unfertilized eggs, floating off the bottom with the slightest disturbance.

DEVELOPMENT OF THE EMBRYO

In discussing the development of yellowfin menhaden eggs the following three stages are used (Ahlstrom and Counts, 1955):

Early—from fertilization to closure of the blastopore.

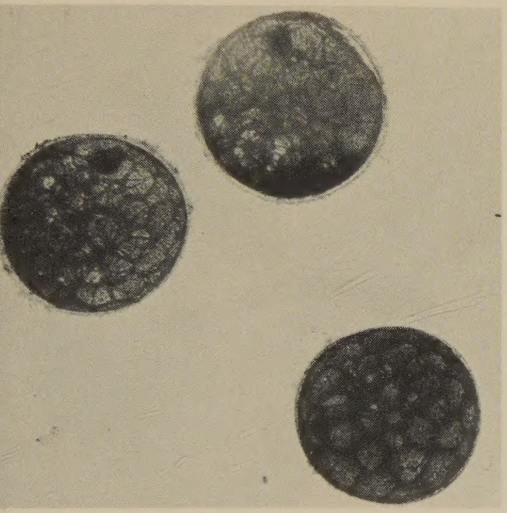
Middle—from closure of the blastopore to the time that the separating tail begins to curve laterally away from the embryonic axis.

Late—from the time the tail curves away from the embryonic axis to the time of hatching.

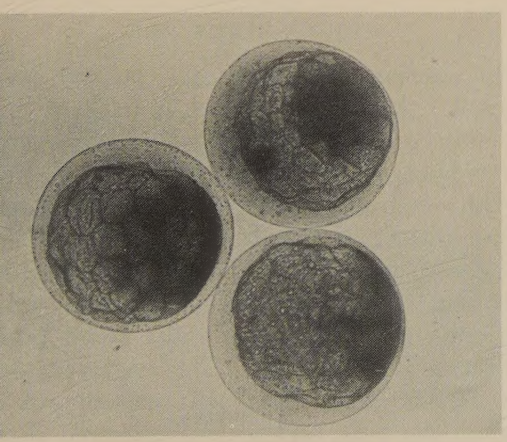
EARLY-STAGE EGG

e perivitelline space developed and widened
n 15 minutes after ova and sperm were mixed
a water. If the ova and sperm were mixed
e absence of water, the perivitelline space
not readily apparent until after sea water
been added. Unfertilized and fertilized eggs
the same lot, one hour after the sex products
mixed, are shown in figures 4 and 5.

rly cleavage was rapid, and a layer of cells
formed by the 7-hour stage (fig. 6). Con-
d cell division resulted in the formation of a
-shaped blastodermal cap on the yolk
7) after 12 hours. Eggs collected from the
kton (fig. 8) showed the blastodermal cap
ing nearly one-third of the yolk. These
blastula were estimated as 14 hours old.



URE 4.—Unfertilized egg of yellowfin menhaden.



URE 5.—One-hour stage with perivitelline space.

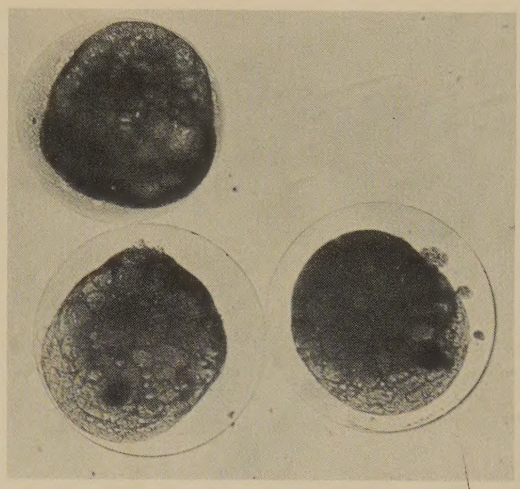


FIGURE 6.—Seven-hour stage with early cleavage.

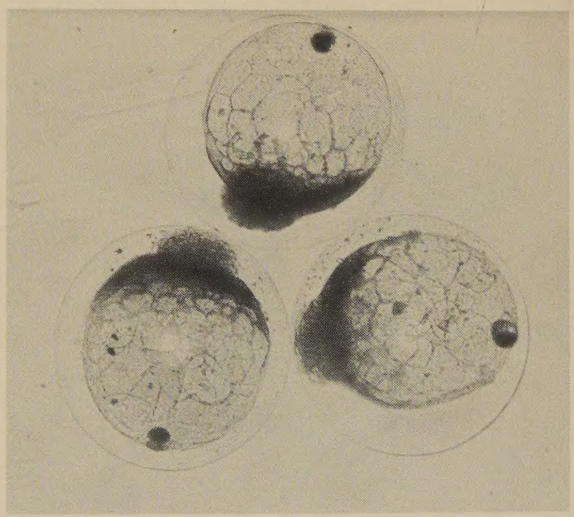


FIGURE 7.—Twelve-hour stage with blastodermal cap.

Some of the early stages showed yolk diffusion into the perivitelline space (fig. 9). This was assumed to be due to mechanical rupture of the yolk membrane during the handling and preservation of the eggs, since yolk encircled by the blastoderm in later stages did not appear to be ruptured (figs. 10, 11, 12, and 15).

At the late blastula stage the blastodermal cap, now known as the embryonic shield (fig. 9), had developed. The early embryo could be seen as a medial thickening of the shield. The peripheral cells continued to spread over the yolk surface.

The early neurula marked the end of the early-stage egg (fig. 10). The developing embryo, with a discernible head and several myomeres, became

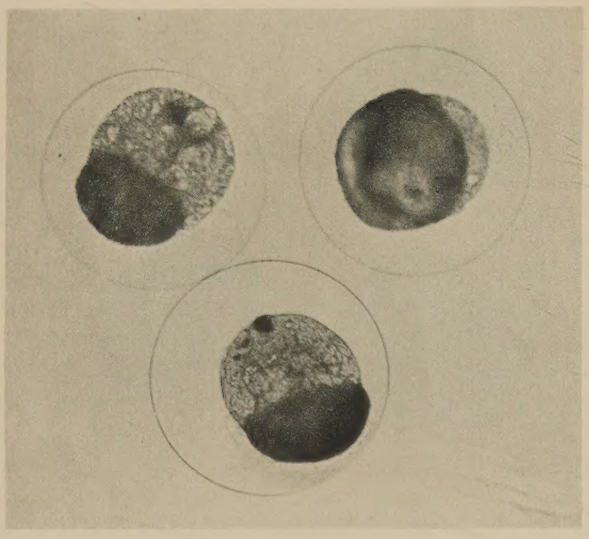


FIGURE 8.—Circa 14-hour stage with blastodermal cap.

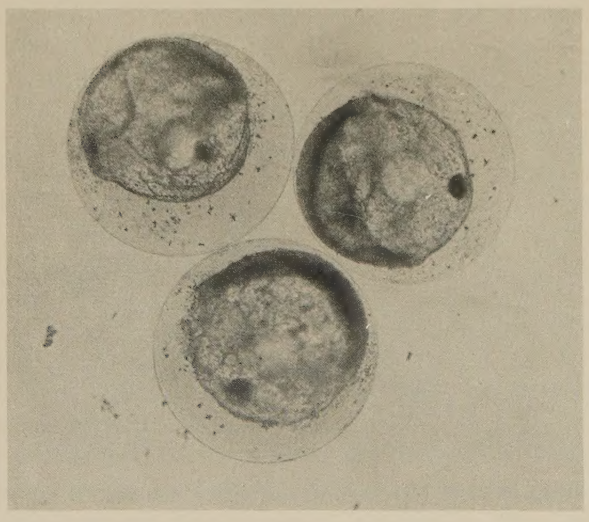


FIGURE 9.—Sixteen-hour stage with embryonic shield.

visible about the time of blastopore closure. Artificially fertilized eggs were not sampled at this stage. Eggs estimated at the 24- and 30-hour stages were obtained from plankton collections made during the rearing studies. Particles adhered to the surface of artificially reared eggs, probably due to the absence of water movement in the culture bowls. Eggs from the plankton were clean by comparison.

The early-stage eggs showed little pigmentation. A few small chromatophores were scattered over the surface of the yolk, but none was apparent on the blastula or early neurula.

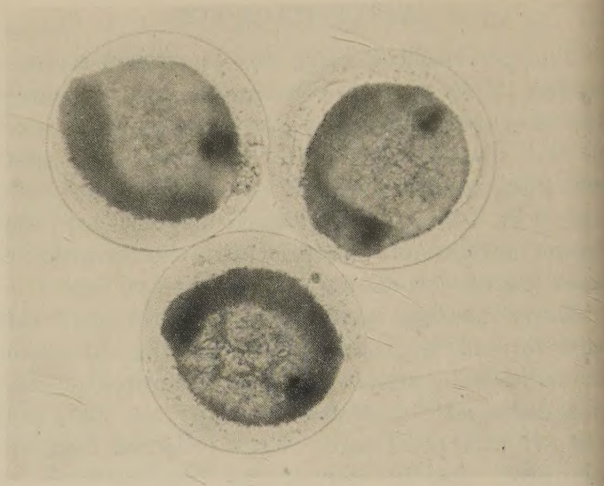


FIGURE 10.—Circa 24-hour stage from plankton with early neurula.

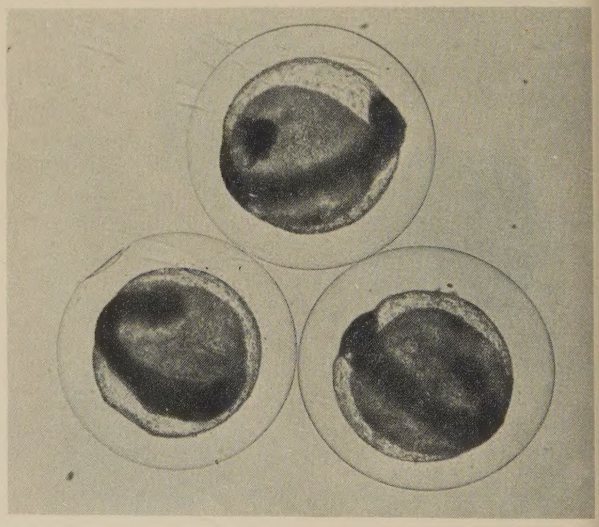
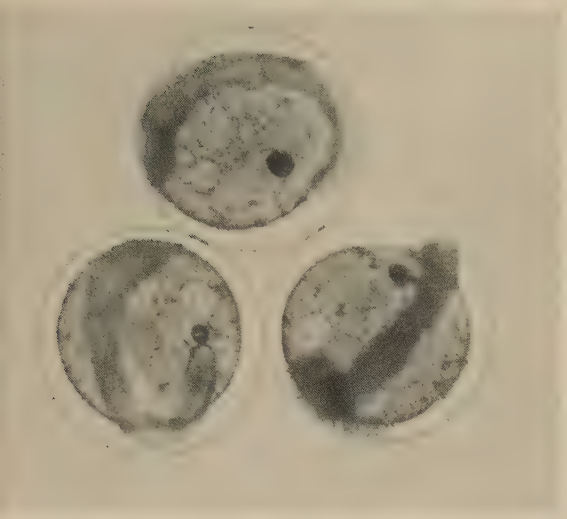


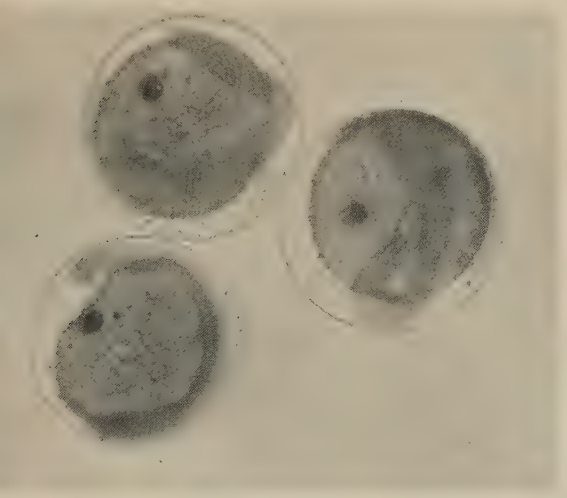
FIGURE 11.—Circa 30-hour stage from plankton with late neurula.

MIDDLE-STAGE EGG

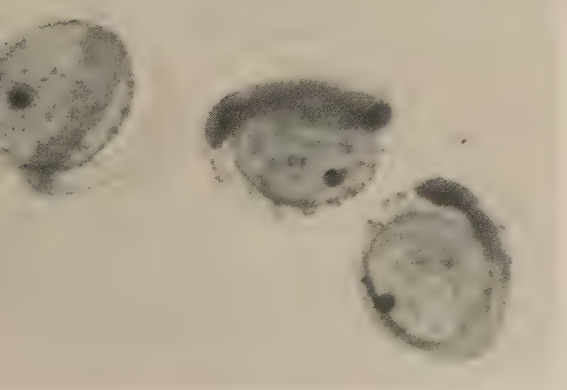
The developing embryo encircled two-thirds of the yolk. Myomeres were visible along most of the embryo, the head was well-defined, and the optic lobes appeared as lateral expansions (fig. 11). The late neurula was raised above the yolk as a cylindrical embryo and not as a mere thickening of the embryonic shield (fig. 12). At the end of this stage, the tail had become separated from the yolk and was curved laterally away from the embryonic axis (fig. 13). This occurred 40 hours after fertilization. Small chromatophores developed on the yolk, and several appeared along the embryo, usually just posterior to the head.



URE 12.—Thirty-eight-hour stage with early embryo.



RE 13.—Forty-one-hour stage with tail separating from yolk.



GURE 14.—Forty-six-hour stage with late embryo.

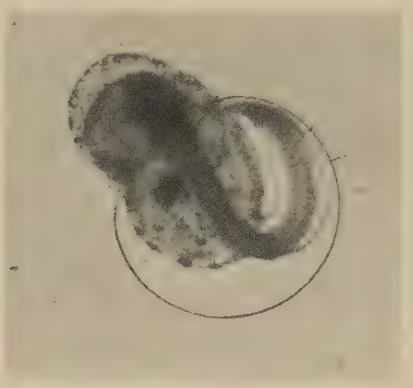


FIGURE 15.—Forty-six-hour stage hatching.

LATE-STAGE EGG

The embryo had grown so large that the tail, free of the yolk, fell just short of touching the head (fig. 14). The somites were visible except near the end of the tail. The embryo was very active, exhibiting convulsive movements at frequent intervals. The tail was outlined with a distinct finfold. Pigmentation generally was limited to one to three small chromatophores along the tail and three to eight in the head or anterior region. Pigmentation of embryos was variable, and no pattern or concentration of chromatophores was discernible. Hatching occurred with the rupture of the external membrane, and the larva emerged, head first (fig. 15), 46 hours after fertilization.

YOLK-SAC LARVA

Yellowfin menhaden, like many other fishes with pelagic eggs (Ahlstrom and Counts, 1955), hatched in a relatively undeveloped condition. The mouth had not formed, and the eyes were unpigmented. Fin rays had not developed, and the pectoral fin buds were not visible. However, the anus had formed and was discernible as a tube passing through the finfold.

The early larva (figs. 16 and 17) floated ventral side up, with the yolk and oil globule uppermost, except during brief, convulsive swimming. During initial swimming, the larva oriented dorsal side up and then, in a head-down position, would move towards the bottom. Body movement would stop after a few seconds and the larva would turn ventral side up and float towards the surface. Such behavior was most marked during the first 24 hours. As the larva grew and the yolk diminished, swimming increased and by 48 hours was

nearly continuous. Even during brief periods of rest, vertical stability was maintained with the dorsal surface up.

Measurements of larvae are given in table 2. The larvae nearly doubled in length during the absorption of the yolk; however, 80 percent of this increase occurred during the first 27 hours.

The late larva continued to lengthen slightly after the 27-hour stage (fig. 18). Between 40 and 60 hours the most apparent change was the shrinking yolk sac (figs. 19 and 20). At the 62-hour stage, eye pigment developed, and the mouth opened (fig. 19). Swimming was continuous and directed as the larva moved across a 6-inch culture bowl with apparent ease. It constantly counteracted the bouyancy of the yolk by swimming in a head-down position. Prior to the development of eye pigment, larvae appeared randomly distributed in the culture bowl and did not react to the approach of the pipette used to collect samples. After the appearance of pigment, larvae oriented away from the source of light and swam from the approaching pipette.

Pigmentation of yolk-sac larvae was limited to widely spaced, small chromatophores along the sides and on the finfold. The chromatophores appeared as faint speckling at a magnification of 20 × and as distinct structures at 100 ×.

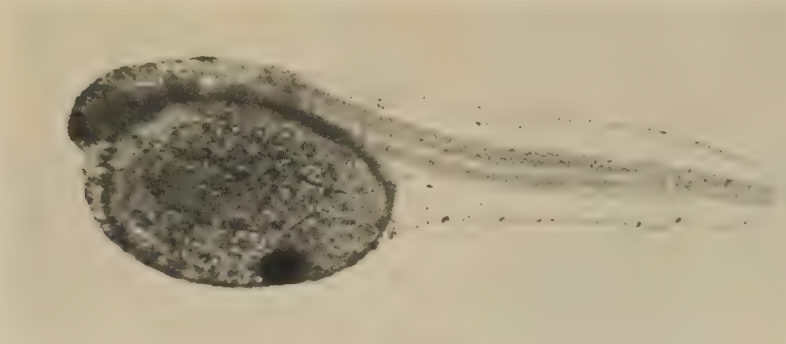


FIGURE 16.--Newly hatched larva 2.8 millimeters long.

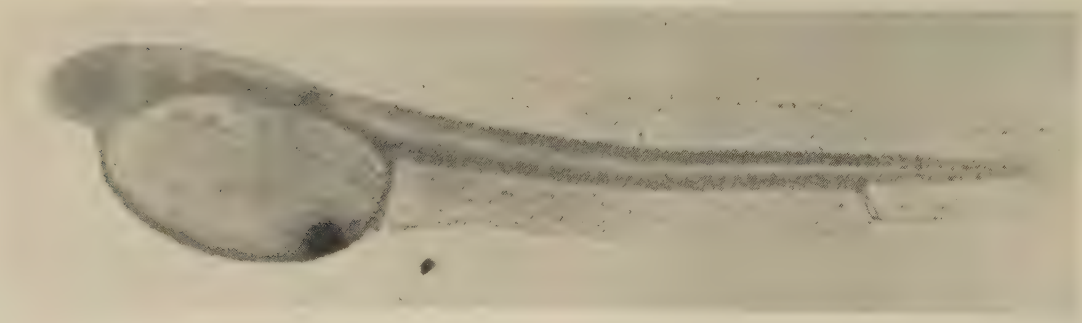


FIGURE 17.—Sixteen-hour larva 4.0 mm. long.

TABLE 2.—*Measurements* [1] *of yellowfin menhaden yolk-sac larvae, in millimeters*

(N=10 specimens at each age)

Hours since hatching	Total length		Distance snout to anus		Yolk-sac length		Yolk-sac width	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0	2.70–2.92	2.79	2.26–2.42	2.35	1.10–1.21	1.16	0.77–.94	0.83
16	3.85–4.18	4.03	3.24–3.41	3.32	1.10–1.21	1.16	.72–.77	.74
27	4.24–4.68	4.46	3.41–3.68	3.59	.94–1.04	1.00	.66–.72	.67
40	4.34–4.62	4.49	3.46–3.74	3.57	.77– .88	.81	.50–.60	.54
62	4.43–5.28	4.86	3.58–4.18	3.79	.38– .60	.53	.22–.39	.27

[1] Measurements of yolk-sac larvae were of preserved material. Ahlstrom and Ball (1954) estimated as much as 20 percent shrinkage due to formalin preservation. Investigators examining fresh larvae should interpret the measurements accordingly.

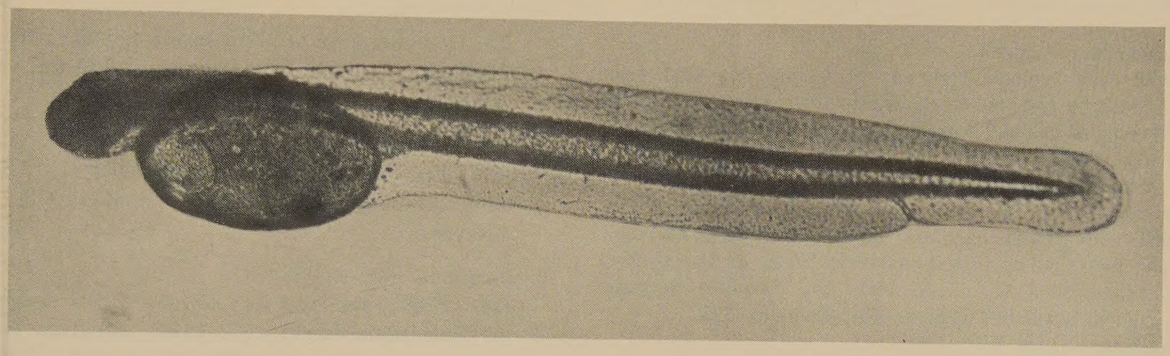


FIGURE 18.—Twenty-seven-hour larva 4.5 mm. long.

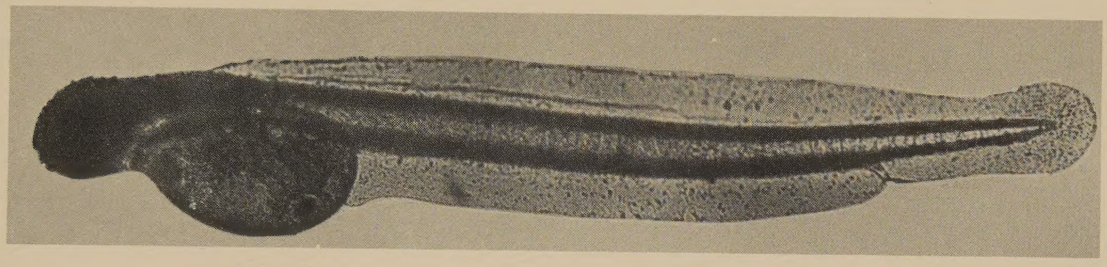


FIGURE 19.—Forty-hour larva 4.5 mm. long.

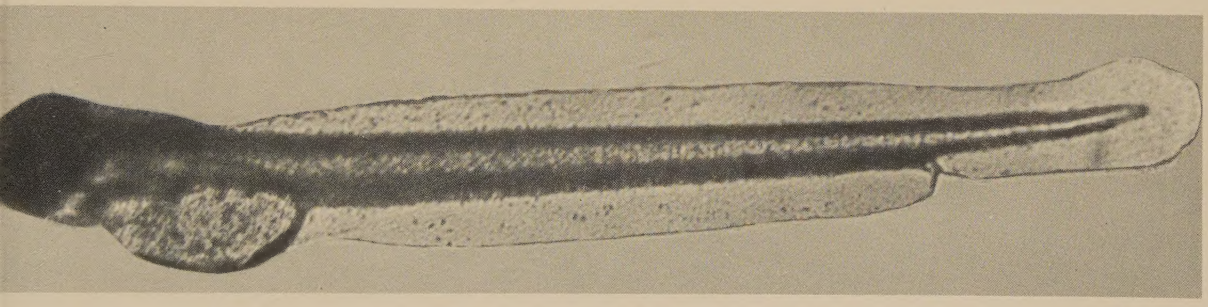


FIGURE 20.—Sixty-two-hour larva 4.9 mm. long.

ıe rest of the larvae died within a few hours the 62-hour stage.

wish to acknowledge the facilities and help shed by Sembler Fisheries, Sebastian, Fla. ons connected with the firm gave direct as-nce during regular and trial fishing trips, kton tow-net collections, and examination of andings.

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